Role of IL-6 in the Pathogenesis of Type 2 Diabetes Mellitus

Thesis

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“No one is as blind as those who have sight without vision.”

–Helen Keller,

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For never giving up, and for teaching me never to give up,

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For helping me become a better human being:

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Abstract

There has been growing evidence that low grade activation of the immune system plays a role in the pathogenesis of insulin resistance and type 2 diabetes mellitus. This study is based on the hypothesis that a single nucleotide polymorphism in the promoter region of Interleukin-6 gene influences its transcription and thus affects plasma level of Interleukin-6.This will in turn lead to insulin resistance and consequently type 2 diabetes.

This study was carried out in Kasr Al-Ainy Hospital on 60 subjects: 30 known diabetic patients (15 obese and 15 lean) and 30 non-diabetic subjects (15 obese and 15 lean).

In the current study, serum level of Interleukin-6 was assayed by enzyme chemiluminescence and -174 G/C gene polymorphism was detected by PCR-RFLP.

Serum level of IL-6 was found to be high in the obese group, both diabetic and non-diabetic and in the lean diabetic group. As for IL-6 genotyping, we found that 58% of the obese carrying the GG genotype developed type 2 diabetes versus 38% of those carrying the C allele. Therefore, this might suggest a protective role for the C allele in the presence of obesity.

Key words: Interleukin-6, Chemiluminescence, -174 G/C polymorphism, PCR-RFLP, Insulin Resistance.

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LIST OF ABBREVIATIONS

ADA	American Diabetes Association
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BMI	Body Mass Index
CABG	Coronary Artery Bypass Graft
cAMP	cyclic Adenosine Monophosphate
CETP	Cholesterol Ester Transfer Protein
ddNTPs	dideoxynucleotides
dNTPs	deoxynucleotides
ELISA	Enzyme-Linked Immunosorbent Assay
FFAs	Free Fatty Acids
GK	Glycerol Kinase
GLDH	Glutamate Dehydrogenase
GLUT	Glucose Transporter
GPO	Glycerol Phosphate Oxidase
HDL	High Density Lipoprotein
HDL-c	High Density Lipoprotein-cholesterol
IAPP	Islet Amyloid Polypeptide
IGT	Impaired Glucose Tolerance
IL-6	Interleukin-6
IL-6R	Interleukin-6 Receptor
IRS-1	Insulin Receptor Substrate-1
LD	Linkage Disequilibrium
LDH	Lactate Dehydrogenase
LDL	Low Density Lipoprotein
LPL	Lipoprotein Lipase

LPS	Lipopolysaccharides
MCP-1	Monocyte Chemoattractant Protein-1
MDH	Malate Dehydrogenase
MODY	Maturity Onset Diabetes of the Young
mRNA	messenger RNA
mtDNA	mitochondrial DNA
MW	Molecular Weight
NEFAs	Non Esterified Fatty Acids
NIH	National Institutes of Health
OGTT	Oral Glucose Tolerance Test
PCR	Polymerase Chain Reaction
PI3-K	Phosphatidylinositol 3-kinase
PPAR-γ	Peroxisome Proliferator Activated Receptor-gamma
RFLP	Restriction Fragment Length Polymorphism
RIA	Radioimmunoassay
sIL-6R	soluble Interleukin-6 Receptor
SNP	Single Nucleotide Polymorphism
SSCP	Single-Strand Conformation Polymorphism
TG	Triglycerides
TGF-β	Transforming Growth Factor-beta
TNF-α	Tumor Necrosis Factor-alpha
VLDL	Very Low Density Lipoprotein
WC	Waist Circumference
WHO	World Health Organization
WHR	Waist Hip Ratio

INTRODUCTION

AND AIM OF THE STUDY

The field of cytokines has expanded tremendously over the past two decades. Initially, thought to be the products of the immune system alone that had immune and hematological functions only. Yet, it has become increasingly apparent that cytokines participate in a neuro-endocrine and immune system network ***(Dimitris and Papanicolaou, 2000)***.

There has been growing evidence that type 2 diabetes mellitus is associated with a subclinical inflammation and that chronic low-grade activation of the immune system plays a role in the pathogenesis of insulin resistance and type 2 diabetes. Although it is well established that insulin resistance and impaired insulin secretion are central to the pathogenesis of type 2 diabetes, it has been unclear how these abnormalities arise and how they are related to the many different clinical and biochemical features common in type 2 diabetes. Activation of innate immunity provides a new model for the pathogenesis of type 2 diabetes, which may explain some or all of these features ***(Pickup, 2004)***.

According to ***Fernández-Real and Ricart*** in ***2003,*** adipose tissue expression and circulating IL-6 concentrations are positively correlated with obesity, impaired glucose tolerance, and insulin resistance. In addition to this, they stated that 1/3 of the circulating IL-6 has been shown to arise from adipose tissue, particularly visceral fat.

The high rate of plasma clearance of IL-6 suggests that IL-6 concentration is regulated mainly on the levels of transcription and translation ***(Illig et al, 2004)***. Therefore, the discovery of single nucleotide polymorphisms (SNPs) in the promoter region of IL-6 gene might be considered as risk factors for the development of type 2 diabetes ***(Vozarova et al, 2003)***. ***Fishman et al*** in ***1998*** identified a single base change (G→C) polymorphism at position -174 in the promoter region of IL-6 gene and stated that this polymorphism is of functional significance. The -174 G/C polymorphism has been reported as functionally important since it influences the transcriptional rate of the gene and in turn plasma concentrations of circulating IL-6 ***(Bennermo, 2005).***

The aim of this study is to find out the role of IL-6 in the pathogenesis of type 2 diabetes mellitus. IL-6 gene polymorphism -174 G/C was also determined to see whether this single nucleotide polymorphism affects IL-6 levels and consequently its role in type 2 diabetes mellitus.

Type 2 Diabetes Mellitus
An Overview

Introduction:

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the ß-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia. ***(American Diabetes Association, 2008)***.

Type 2 diabetes, which accounts for ~90–95% of those with diabetes, was previously referred to as non-insulin-dependent diabetes, or adult-onset diabetes, and it encompasses individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency. Although the specific etiologies are not known, autoimmune destruction of ß-cells does not occur, which is the cause of most cases of type 1 diabetes ***(American Diabetes Association, 2008)***.

A link between obesity and type 2 diabetes has long been recognized. About 80% of patients are obviously obese at the time of diagnosis, usually with a central fat distribution in and around the abdominal cavity. In addition, many of those who are not obese by traditional weight criteria have an increased percentage of body fat, distributed predominantly in the abdominal region. Obesity is the main risk factor underlying the pandemic of type 2 diabetes and is also the most obvious target for measures to prevent type 2 diabetes ***(Katsilambros and Tentolouris, 2003)***.

Natural History of Type 2 Diabetes:

Type 2 diabetes is predicted by multiple traits, among which are obesity, visceral fat accumulation, insulin resistance and hyperinsulinemia itself ***(Hanley et al, 2003)***. In the individual who is destined to become diabetic, the factors that control glucose tolerance all must be more or less altered, generating a critical state of instability. In such a condition, phase transition can be triggered by relatively small further changes and occur relatively rapidly. In the case of glucose tolerance, transition from obese, insulin-resistant to overt diabetes may take the form of a large, rapid rise in glucose levels as a result of further loss of ß-cell competence ***(Ferrannini et al, 2004)***.

Initially, insulin resistance is compensated for by the adaptive capacity of the ß-cells to increase insulin concentrations, thus preventing any serious disturbance in glucose homeostasis. Ultimately, whether through worsening insulin resistance or progressive impairment of ß-cell function, insulin secretion reaches a plateau, during which blood glucose